

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

**1 – 51 (Cancelled).**

**52 (Currently amended).** A method of producing in a eukaryotic cell at least one protein comprising at least one unnatural amino acid, the method comprising:

growing, in an appropriate medium, a eukaryotic cell that comprises a nucleic acid that comprises at least one selector codon and encodes the protein; wherein the medium comprises an unnatural amino acid and the eukaryotic cell comprises:

an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon;  
and,

an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS comprises an amino acid sequence that corresponds to any one of SEQ ID NOs[[.]]: 48 – 53.

**53 (Currently amended).** A method of producing in a eukaryotic cell at least one protein comprising at least one unnatural amino acid ~~and modifying said protein~~, the method comprising:

growing, in an appropriate medium, a eukaryotic cell that comprises a nucleic acid that comprises at least one selector codon and encodes the protein; wherein the medium comprises the unnatural amino acid and the eukaryotic cell comprises an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon and an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS is ~~selected from~~:

(i) a *p*-propargyloxyphenylalanine O-RS that preferentially aminoacylates the O-tRNA with a *p*-propargyloxyphenylalanine, ~~and~~ or

(ii) a *p*-azido-L-phenylalanine O-RS that preferentially aminoacylates the O-tRNA with a *p*-azido-L-phenylalanine, the *p*-azido-L-phenylalanine O-RS comprising :

(i) an amino acid sequence set forth in any one of SEQ ID NOs: 48 – 53 ~~and~~ or a conservative variant[[s]] thereof, wherein the conservative variant[[s are]] is at least 98% identical to any one of SEQ ID NOs: 48 – 53, or

(ii) an amino acid sequence that is a conservative variant of SEQ ID NO: 2, which conservative variant is at least 98% identical to SEQ ID NO: 2 and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37; aspartate at a position corresponding to Asn126; asparagine at a position corresponding to Asp182; alanine, or valine, at a position corresponding to Phe183; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186; and,

incorporating into the protein the unnatural amino acid in the eukaryotic cell, wherein the unnatural amino acid comprises a first reactive group; ~~and,~~

~~contacting the protein with a molecule that comprises a second reactive group; wherein the first reactive group reacts with the second reactive group to attach the molecule to the unnatural amino acid through a [3+2] cycloaddition, thereby modifying the protein.~~

**54 (Currently amended).** The method of claim ~~[[53]] 65~~, wherein the molecule is a dye, a polymer, a derivative of polyethylene glycol, a photocrosslinker, a cytotoxic compound, an affinity label, a derivative of biotin, a resin, a second protein or polypeptide, a metal chelator, a cofactor, a fatty acid, a carbohydrate, or a polynucleotide.

**55 (Cancelled).**

**56 (Currently amended).** The method of claim ~~[[53]] 65~~, wherein the O-RS is the *p*-propargyloxyphenylalanine O-RS, the unnatural amino acid is *p*-propargyloxyphenylalanine, the first reactive group is an alkynyl moiety and the second reactive group is an azido moiety.

**57 (Previously presented).** The method of claim 53, wherein the unnatural amino acid comprises a *p*-propargyloxyphenylalanine.

**58 (Currently amended).** The method of claim ~~[[53]] 65~~, wherein the O-RS is the *p*-azido-L-phenylalanine O-RS, the unnatural amino acid is *p*-azido-L-phenylalanine, the first reactive group is an azido moiety, and the second reactive group is an alkynyl moiety.

**59 (Previously presented).** The method of claim 53, wherein the unnatural amino acid comprises a *p*-azido-L-phenylalanine.

**60 – 61 (Cancelled).**

**62 (Withdrawn).** The method of claim 53, wherein the O-RS is the *p*-propargyloxyphenylalanine O-RS, which *p*-propargyloxyphenylalanine O-RS comprises an amino acid sequence set forth in anyone of SEQ ID NOs: 54-63, and conservative variants thereof, which conservative variants are at least 90% identical to that of a. naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprise two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of *E. coli* TyrRS; aspartate at a position corresponding to Asn126 of *E. coli* TyrRS; asparagine at a position corresponding to Asp182 of *E. coli* TyrRS; alanine or valine at a position corresponding to Phe183 of *E. coli* TyrRS, and methionine, valine, cysteine, or threonine at a position corresponding to Leu186 of *E. coli* TyrRS.

**63 (Previously presented).** A method of producing in a yeast cell at least one protein of interest comprising at least one unnatural amino acid selected from *p*-azido-L-phenylalanine and *p*-propargyloxyphenylalanine, the method comprising growing, in an appropriate medium, the yeast cell, wherein the medium comprises the unnatural amino acid, and wherein the yeast cell comprises:

- (a) a nucleic acid encoding said protein of interest and comprising at least one selector codon;
- (b) an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon, wherein the O-tRNA is derived from an *Escherichia coli* tRNA; and,
- (c) an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the unnatural amino acid, wherein the O-RS is derived from an *Escherichia coli* aminoacyl tRNA synthetase.

**64 (Cancelled).**

**65 (New).** The method of claim 53, wherein the method further comprises contacting the protein with a molecule that comprises a second reactive group; wherein the first reactive group reacts with the second reactive group to attach the molecule to the unnatural amino acid through a [3+2] cycloaddition, thereby modifying the protein